J Clin Periodontol 2010; 37: 353–365 doi: 10.1111/j.1600-051X.2010.01538.x

Journal of Clinical Periodontology

# Short-term benefits of the adjunctive use of metronidazole plus amoxicillin in the microbial profile and in the clinical parameters of subjects with generalized aggressive periodontitis

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*Mestnik MJ, Feres M, Figueiredo LC, Duarte PM, Lira EAG, Faveri M. Short-term benefits of the adjunctive use of metronidazole plus amoxicillin in the microbial profile and in clinical parameters of subjects with generalized aggressive periodontitis. J Clin Periodontol 2010; 37: 353–365. doi: 10.1111/j.1600-051X.2010.01538.x*

***Abstract***

**Aim:** The aim of this study was to evaluate the clinical and microbiological effects of scaling and root planing (SRP) alone or combined with metronidazole (MTZ) and amoxicillin (AMX) in the treatment of subjects with generalized aggressive periodontitis (GAgP).

**Materials and Methods:** A double-blind, placebo-controlled, randomized clinical trial was conducted in 30 subjects receiving SRP alone or combined with MTZ (400 mg 3 × per day) and AMX (500 mg 3 × per day) for 14 days. Clinical and microbiological examinations were performed at baseline and 3 months post-SRP. Nine subgingival plaque samples per subject were analysed using checkerboard DNA–DNA hybridization.

**Results:** Subjects receiving MTZ and AMX showed the greatest improvements in the mean full-mouth probing depth and clinical attachment level and at initially intermediate and deep sites. The most beneficial changes in the microbial profile were also observed in the MTZ+AMX group, which showed the lowest proportions of the red complex as well as a significant decrease in the proportions of the orange complex after treatment. The antibiotic therapy also reduced the levels of *Aggregatibacter actinomycetemcomitans* at initially deep sites.

**Conclusion:** Subjects with GAgP significantly benefit from the adjunctive use of MTZ and AMX. The short-term advantages are observed in the clinical and microbiological parameters.

Key words: dental plaque; generalized aggressive periodontitis; periodontal disease; scaling and root planing

Accepted for publication 10 December 2009

**Conflict of interest and source of funding statement**

The authors declare that they have no conflict of interests.

This study was supported in part by Research Grants 2007/56413-0 from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil).

Aggressive periodontitis (AgP) is characterized by a rapid loss of clinical attachment and alveolar bone and normally affects subjects younger than 30 years old (Ranney 1993, Albandar et al. 1997, Lang et al. 1999, Tonetti & Mombelli 1999). These infections are subdivided into localized or generalized, according to the extent of the periodontal destruction (Armitage 1999, Lang et al. 1999, Lindhe et al. 1999). As opposed to chronic periodontitis, the amount of biofilm and calculus accumulation in AgP subjects is inconsistent with the severity and progression of the periodontal destruction (Lang et al. 1999).

The benefits of systemic antibiotics in the treatment of AgP have recently been reported in two systematic reviews (Herrera et al. 2002, Haffajee et al. 2003); however, the most effective agents and treatment protocols are still to be defined. The association of metronidazole (MTZ) and amoxicillin (AMX) in the treatment of AgP has gained recognition mostly due to its effectiveness against *Aggregatibacter actinomycetemcomitans* (van Winkelhoff et al. 1989, Pavicić et al. 1992, 1994, Winkel et al. 2001), a periodontal pathogen closely associated with the aetiology of these infections. However, recent reports have demonstrated that other periodontal pathogens such as *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*, *Campylobacter rectus*, as well as *Prevotella* and *Selenomonas* species may have an important role in the aetiology and progression of AgP (Mullally et al. 2000, Gajardo et al. 2005, Ximenez-Fyvie et al. 2006, Thiha et al. 2007, Faveri et al. 2008, 2009). In addition, a recent report from our group (Faveri et al. 2009) showed that the proportions of the host-compatible *Actinomyces* species might be reduced in these subjects. Therefore, effective treatment of AgP would involve not only a reduction in periodontal pathogens but also an increase in the *Actinomyces* species. However, the effect of different antibiotic regimens, including the association of MTZ and AMX with scaling and root planing (SRP), in changing the microbial profile of AgP subjects has not yet been investigated.

Interestingly, the first randomized placebo-controlled clinical trial (RCT) that evaluated the clinical effects of MTZ and AMX combined with SRP was only recently published (Guerrero et al. 2005). The authors showed that this treatment led to a better clinical response than that observed with SRP alone. Afterwards, the benefits of this combination of drugs in the clinical parameters of generalized aggressive periodontitis (GAgP) subjects were corroborated by two other reports (Kaner et al. 2007, Machtei & Younis 2008). However, no microbiological data from these studies were described. In fact, so far, only one RCT has evaluated the effect of this antibiotic regimen on four periodontal pathogens in the treatment of GAgP subjects (Xajigeorgiou et al. 2006). The authors reported that the adjunctive use of MTZ plus AMX to SRP significantly reduced the levels of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* in deep pockets of subjects with GAgP for 6 months post-SRP.

Given that no studies to date have thoroughly evaluated the changes that occur in the subgingival microbial profile when systemic antibiotics are used as part of the periodontal treatment of subjects with GAgP, the aim of the present study was to evaluate the clinical and microbiological effects of SRP combined with systemic MTZ and AMX in the treatment of GAgP.

## Materials and Methods

### Sample size calculation

The ideal sample size to assure adequate power for the microbiological data of this clinical trial was calculated considering differences of at least 6.6% between groups for the proportion of the red complex species and a standard deviation of 5.0% (Matarazzo et al. 2008). It was determined that 13 subjects per group would be necessary to provide 80% power with an $\alpha$ of 0.05. Based on the mean drop-out rate of 15% from our previous studies, 15 subjects were included in each group.

### Subject population

Subjects recruitment started in July 2007 and was completed by the end of September 2008. Thirty subjects with previously untreated periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University (Guarulhos, SP, Brazil). Detailed medical and dental histories were obtained. Subjects who fulfilled the inclusion criteria were invited to participate in the study. All eligible subjects were thoroughly informed of the nature, potential risks and benefits of their participation in the study and signed a Term of Informed Consent. This study protocol was approved previously by Guarulhos University Ethics Committe in clinical research.

### Inclusion and exclusion criteria

All subjects were in good general health and had presented with at least 20 teeth excluding third molars and teeth indicated for extraction. All subjects were diagnosed with GAgP, based on the current International classification of the American Academy of Periodontology (Armitage 1999). The inclusion criteria were as follows:

- $\leqslant$30 years of age;
- Minimum of six permanent teeth including incisors and/or first molars with at least one site each with probing depth (PD) and clinical attachment level (CAL) $\geqslant$5 mm and a minimum of six teeth other than first molars and incisors with at least one site each with PD and CAL $\geqslant$5 mm; and
- Familial aggregation (during the anamneses, the subjects were asked whether they had at least one other member of the family presenting or with a history of periodontal disease).

The exclusion criteria were as follows: previous subgingival periodontal therapy, smoking, pregnancy, systemic diseases that could affect the progression of periodontal disease (e.g. diabetes and immunological disorders), long-term administration of anti-inflammatory medication, need for antibiotic coverage for routine dental therapy, antibiotic therapy in the previous 6 months and allergy to chlorhexidine (CHX), AMX and MTZ.

### Experimental design

In this double-blinded, RCT, subjects were randomly assigned using a computer-generated table to one of the following treatment groups: Control (C): SRP +Placebo and Test (T): SRP+systemic MTZ at the dosage of 400 mg and AMX at the dosage of 500 mg. Subjects in the Control group received MTZ and AMX placebos. Both antibiotics and placebos were administered 3 × per day for 14 days. Supragingival biofilm control in both groups was achieved by rinsing with a 0.12% CHX solution. All subjects were instructed to gargle with 15 ml of CHX twice a day for 60 days for 1 min., i.e., in the morning, 30 min. after breakfast and tooth brushing, and at night, before going to sleep. The antibiotic or placebo therapies and the CHX rinses started immediately after the first session of mechanical instrumentation. Guarulhos University Pharmacy prepared the antibiotic and placebo capsules for 30 subjects. One hundred and twenty identical opaque plastic packs with 21 capsules (30 packs with MTZ 400 mg, 30 with AMX 500 mg, 30 MTZ placebo and 30 AMX

placebo) were sent to the study coordinator (M. Fa.), who marked the code number of each subject on a set of two packs, according to the therapy assigned. All the capsules in the placebo and antibiotics packs were identical. The coded packs were given to the examiner (M. J. M.), who at no time during the study had any access to information about the contents of the tubes or the assignment of subjects to the two therapies. In addition, all study personnel, including the biostatisticians and participants, were blinded to treatment assignment during the study.

Before the study began, all subjects received full-mouth supragingival scaling and instruction on proper home-care techniques. They were also given the same dentifrice to use during the period of the study (Colgate Total®, Anakol Ind. Com. Ltda- Kolynos do Brasil – Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil). All subjects received full-mouth SRP performed under local anaesthesia in a maximum of six appointments lasting approximately 1 h each. Treatment of the entire oral cavity was completed in a maximum period of 14 days. SRP was performed by one trained periodontist using manual instruments. All subjects received microbiological and clinical monitoring at baseline and at 3 months post-therapy.

#### Compliance and adverse events

The subjects were asked to bring the packs containing the medication once a week when compliance was checked. The packs contained 21 capsules of each placebo or antibiotic, enough for 1 week of medication (21 capsules, 3 × per day for 7 days). During these visits, subjects returned the old pack containing the placebo or the antibiotic and received a new pack of medication/placebo. They also answered a questionanaire about any self-perceived side-effects of the medication/placebo. Two study assistants conducted this inquiry, and were also responsible for calling the subjects every 2 days to monitor compliance.

#### Clinical monitoring

Clinical monitoring was performed by one calibrated examiner (see ''Investigator's Calibration'') and the treatment was carried out by another clinician. Thus, the examiner and the clinician were masked as to the nature of the treatment groups. Subjects were clinically monitored at baseline and at 3 months post-therapy. Visible plaque, gingival bleeding, bleeding on probing (BOP), suppuration, PD (mm) and CAL (mm) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) in all teeth, excluding third molars. First, visible plaque was scored as present if a film of plaque was visible to the naked eye after drying the tooth with a blast air. Gingival bleeding was scored as present or absent by running the probe 1–2 mm into the gingival crevice. Subsequently, the PD and CAL measurements were recorded to the nearest millimetre using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). The cemento-enamel junction was detected by probing the cervical area of each tooth and was used to calculate the CAL. BOP and supuration were recorded as present or absent after PD and CAL measurements.

#### Investigator's calibration

The examiner participated in a calibration exercise that was performed in 10 non-study subjects with periodontitis. The examiner measured one quadrant per subject. The quadrant chosen had at least six teeth. If a quadrant presented fewer than six teeth, a second quadrant was chosen. For better standardization, quadrant #1 was the first choice, followed by #2, #3 and #4, respectively. Initially, the examiner measured PD and CAL in a given quadrant and 60 min. later, this same protocol was repeated. Therefore, all 10 subjects were probed twice in the same visit by the examiner. Upon completion of all measurements, the intra-examiner variabilities for PD and CAL measurements were assessed. Calibration was conducted according to the protocol developed by Araujo et al. (2003), and the standard error of measurement (SE) was calculated. The intra-examiner variability was 0.15 mm for PD and 0.19 mm for CAL.

#### Microbiological monitoring

##### *Sample collection*

Subgingival plaque samples were collected at baseline and at 3 months post-SRP from nine non-contiguous inter-proximal sites per subject. The selected sites were randomized into different quadrants and subsets according to baseline PD, three samples in each of the following categories: shallow (PD $\leqslant$ 3 mm), intermediate (PD 4–6 mm) and deep (PD $\geqslant$ 7 mm). After the clinical parameters had been recorded, the supragingival plaque was removed and the subgingival samples were taken with individual sterile mini-Gracey curettes (#11–12) and immediately placed in separate Eppendorf tubes containing 0.15 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). One hundred microlitres of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer.

##### *Checkerboard DNA–DNA hybridization*

Counts of 40 bacterial species were determined in each sample, using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994, Matarazzo et al. 2008). The microbiological analysis was entirely performed at the Laboratory of Microbiology of Guarulhos University. The samples were boiled for 10 min. and neutralized using 0.8 ml of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot 30 apparatus (Immunetics, Cambridge, MA, USA), concentrated on a 15 × 15 cm positively charged nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking it at 120°C for 20 min. The membrane was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labelled whole genomic DNA probes for 40 bacterial species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes were detected using the antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. The last two lanes in each run contained standards at concentrations of $10^5$ and $10^6$ cells of each species. Signals were evaluated visually by comparison with the standards at $10^5$ and $10^6$ bacterial cells for the test species on the same membrane by a calibrated examiner ($\kappa$ test = 93%). They were recorded as: 0 = not detected; 1 = $<10^5$ cells; 2 = $\sim 10^5$ cells; 3 = $10^5$–$10^6$ cells; 4 = $\sim 10^6$ cells; or 5 = $>10^6$ cells. The sensitivity of this assay was adjusted to allow detection of $10^4$ cells of a given species by adjusting the concentration of each DNA probe.

This procedure was carried out in order to provide the same sensitivity of detection for each species. Failure to detect a signal was recorded as zero, although conceivably, counts in the 1–1000 ranges could have been present.

#### Primary and secondary outcome variables

This study compared the microbiological and clinical effects of two different periodontal therapies. The primary outcome variable of the study was changes in the mean proportion of the red complex species. Secondary outcome variables included differences between therapies for the mean changes in the mean levels and proportion of the 40 bacterial species analysed, changes in the mean percentage of sites colonized by *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola*, changes in the mean full-mouth PD and CAL, as well as in different initial PD categories and the percentage of sites with PD changing from PD $\geqslant$ 5 mm to $<$ 5 mm.

#### Statistical analysis

The mean percentage of sites with visible plaque, gingival bleeding, BOP and suppuration, as well as mean PD and CAL were computed for each subject and then averaged across subjects in both groups. Similarly, the changes in PD, CAL and BOP over time were examined in subsets of sites according to the initial PD of $\leqslant$3, 4–6 and $\geqslant$7 mm. Values for each clinical parameter were averaged separately within the three PD categories in each subject and then averaged across subjects in the treatment groups. The mean counts ($\times 10^5$) of individual bacterial species were averaged within each subject and then across subjects in both groups. The percentage of the total DNA probe counts was determined initially in each site, then per subject and averaged across subjects in the two groups. The prevalence of *A. actinomycetemcomitans*, *T. forsythia*, *P. gingivalis* and *T. denticola* was computed by determining the percentage of sites per subject colonized by each species and then averaging across subjects in the two groups. The significance of differences between the two groups for age and the clinical and microbiological parameters was determined using the Mann–Whitney *U*-test. The Wilcoxon test was used to detect statistically significant differences within each group between the two time points. Adjustments for multiple comparisons (Socransky et al. 1991) were performed when the 40 bacterial species were evaluated simultaneously. The chi-square test was used to compare the differences in the frequency of gender. The level of significance was set at 5%.

## Results

#### Subject retention

There were no drop-outs during the course of the experimental period. All subjects returned for the 3-month follow-up visit. Thus, a total of 30 subjects completed the study, 15 in the Control group (SRP+Placebo) and 15 in the Test group (SRP+MTZ+AMX). Figure 1 presents the flow chart of the study design.

#### Adverse effects and compliance

All subjects who finished the study reported full adherence to the prescribed course of the antibiotic/placebo and the CHX rinses. Two subjects, one from the Test group and one from the Control group, reported adverse events (diarrhoea and vomiting) during the study. All subjects reported that the medications did not cause any major disturbance in their daily routine and that they would start the treatment again if necessary.

#### Microbiological findings

The two groups were microbiologically homogeneous at the beginning of the study. No significant differences were observed between them in the mean counts and proportions of any of the test species at baseline (data not shown, $p > 0.05$). Figure 2 presents the mean counts ($\times 10^5$) of the 40 species evaluated over the course of the study. The species were grouped according to the microbial complexes described by Socransky et al. (1998). In general, the counts of most of the host-compatible species did not change significantly from baseline to 3 months post-therapy (blue, purple, yellow and green complexes). *Actinomyces naelsundi 2* presented a significant increase in levels in both groups and there was a significant reduction in the levels of *Capnocytophaga sputigena* in the Test group. A reduction in the mean counts of several periodontal pathogens from the red and orange complexes was observed, mainly in the Test group ($p < 0.05$). *Eubacterium nodatum* was the only species from the orange complex that was reduced in the Control group, while five species (*C. rectus*, *E. nodatum, Fusobacterium nucleatum ss. nucleatum, Parvimonas micra* and *Prevotella intermedia*) were reduced in the Test group. The counts of the three pathogens from the red complex, *T. forsythia*, *P. gingivalis* and *T. denticola,* were significantly reduced in the Test group, while SRP alone did not significantly affect the levels of *T. denticola* at 3 months ($p < 0.05$). In addition, four bacterial species not grouped into complexes (''Others'') were also significantly reduced by the antibiotic treatment *(Eubacterium saburreum*, *Leptotrichia buccalis*, *Propionibacterium acnes* and *Treponema socranskii*).

Figure 3 presents the mean percentage of the DNA probe counts of the 40 individual species evaluated at baseline and 3 months post-SRP. The proportions of seven periodontal pathogens were significantly reduced in the Test group (*C. rectus*, *E. nodatum*, *F. nucleatum ss nucleatum*, *P. intermedia*, *T. forsythia*, *P. gingivalis* and *T. denticola*), while four periodontal pathogens were reduced in the Control group (*E. nodatum, T. forsythia*, *P. gingivalis* and *T. denticola*). In general, the proportions of the putative periodontal pathogens from the orange complex were not profound affected by SRP alone. There was an overall trend towards increasing proportions of the majority of the host-compatible microorganisms, such as the *Actinomyces* species as well as the purple, yellow and green complexes after treatments, especially in the Test group. These changes were statistically significant for *A. naeslundi 2* and *Streptococcus sanguinis* in both treatment groups, for *Actinomyces odontolyticus* and *Veilonella parvula* in the Test group and for *Streptococcus gordonii* in the SRP group.

Figure 4 shows the changes in the proportions of the microbial complexes in the two groups at baseline and 3 months post-treatments. The microbial profiles were profoundly affected by treatments, and the most beneficial changes were observed in subjects who received MTZ and AMX as part of the treatment. These subjects showed a significant reduction in the proportions of red and orange complexes from baseline

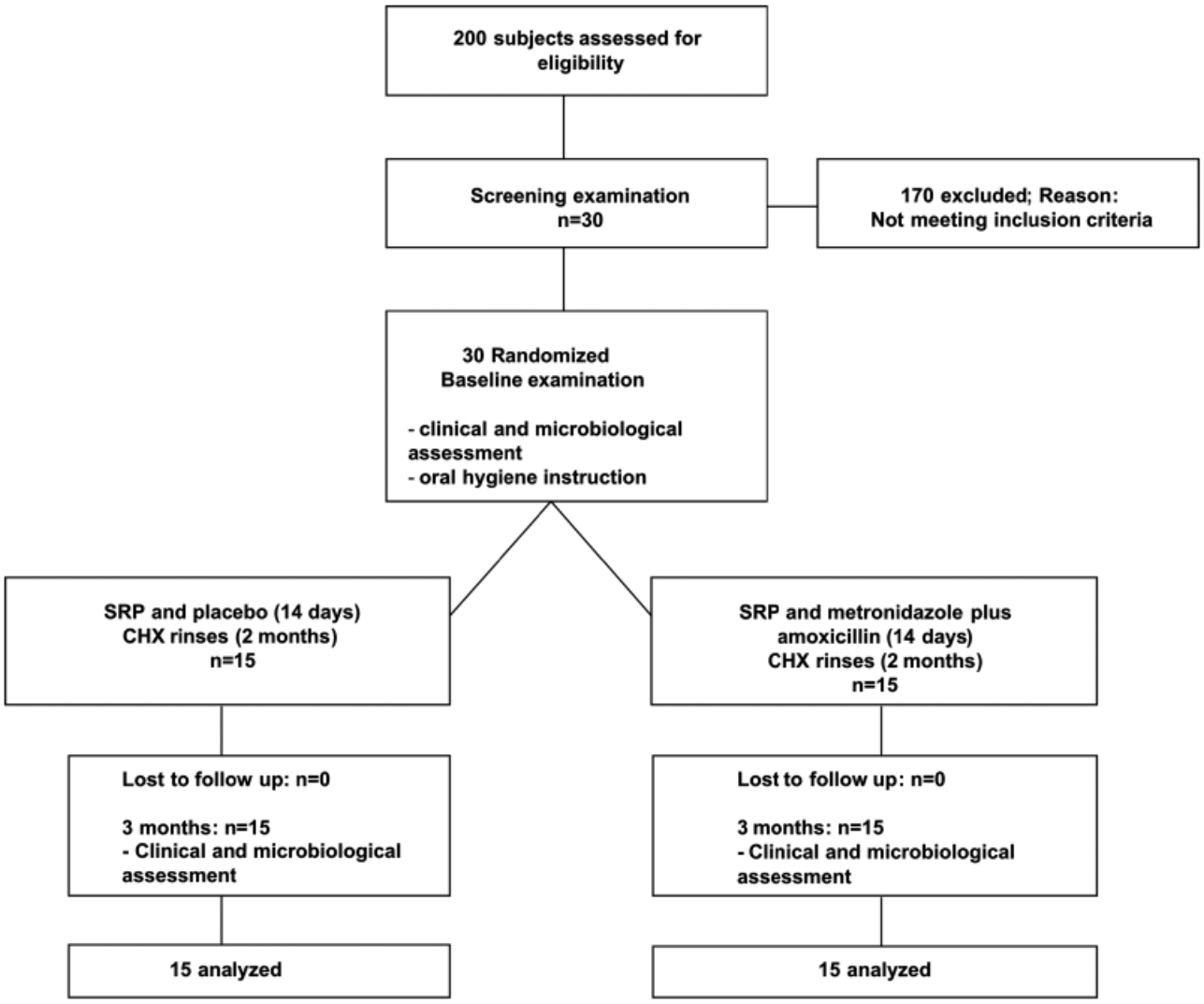


*Fig. 1*. Flow chart of the study design.

to 3 months post-therapy as well as an increase in the proportion of beneficial *Actinomyces* species (blue complex), purple and yellow complexes. SRP alone led to a significant reduction in the proportions of red complex and a significant increase in the proportions of blue, yellow and purple complexes. Even though no differences in the proportions of the microbial complexes were observed between the two groups at baseline, at 3 months post-treatment, there was a significantly lower proportion of the red complex species in the Test group in comparison with the Control group ($p < 0.05$).

Figure 5 presents the mean change between baseline and 3 months post-SRP in the mean counts ($\times 10^5$) of *A. actinomycetemcomitans* according to the initial PD categories ($\leqslant 3$ mm, 4–6 mm and $\geqslant 7$ mm). An overall reduction in the mean counts was observed for both treatments in the three PD categories. However, subjects in the Test group showed a significantly greater reduction in the levels of this pathogen in the initially deep sites ($\geqslant 7$ mm), in comparison with the Control group ($p < 0.05$).

The mean percentages of sites colonized by *A. actinomycetemcomitans* and the red complex species (*T. forsyhtia*, *P. gingivalis* and *T. denticola*) at baseline and at 3 months after treatments are presented in Table 1. Both therapies led to a statistically significant decrease in the mean percentage of sites colonized by these species, with the exception of *A. actinomycetemcomitans* in the Control group ($p > 0.05$). At 3 months post-SRP, the mean percentages of sites colonized by these four bacterial species were significantly lower in the Test group in comparison with the Control group for all the species analysed ($p < 0.05$).

Table 2 presents the frequency of subjects (nine sites per/subject) colonized by *A. actinomycetemcomitans* at baseline and at 3 months post-SRP in the two treatment groups. At baseline, all subjects from the Control and Test groups were colonized by *A. actinomycetemcomitans*. At 3 months after treatment, four subjects in the Test group tested negative for *A. actinomycetemcomitans* and eight subjects presented this species in only one site. On the other hand, in the Control group, only two subjects tested negative for *A. actinomycetemcomitans* after treatment and colonization by this species was shown in two other subjects in only one site. In addition, none of the subjects who took antibiotics showed heavy colonization ($\geqslant 5$ mm sites) by this pathogen after treatment.

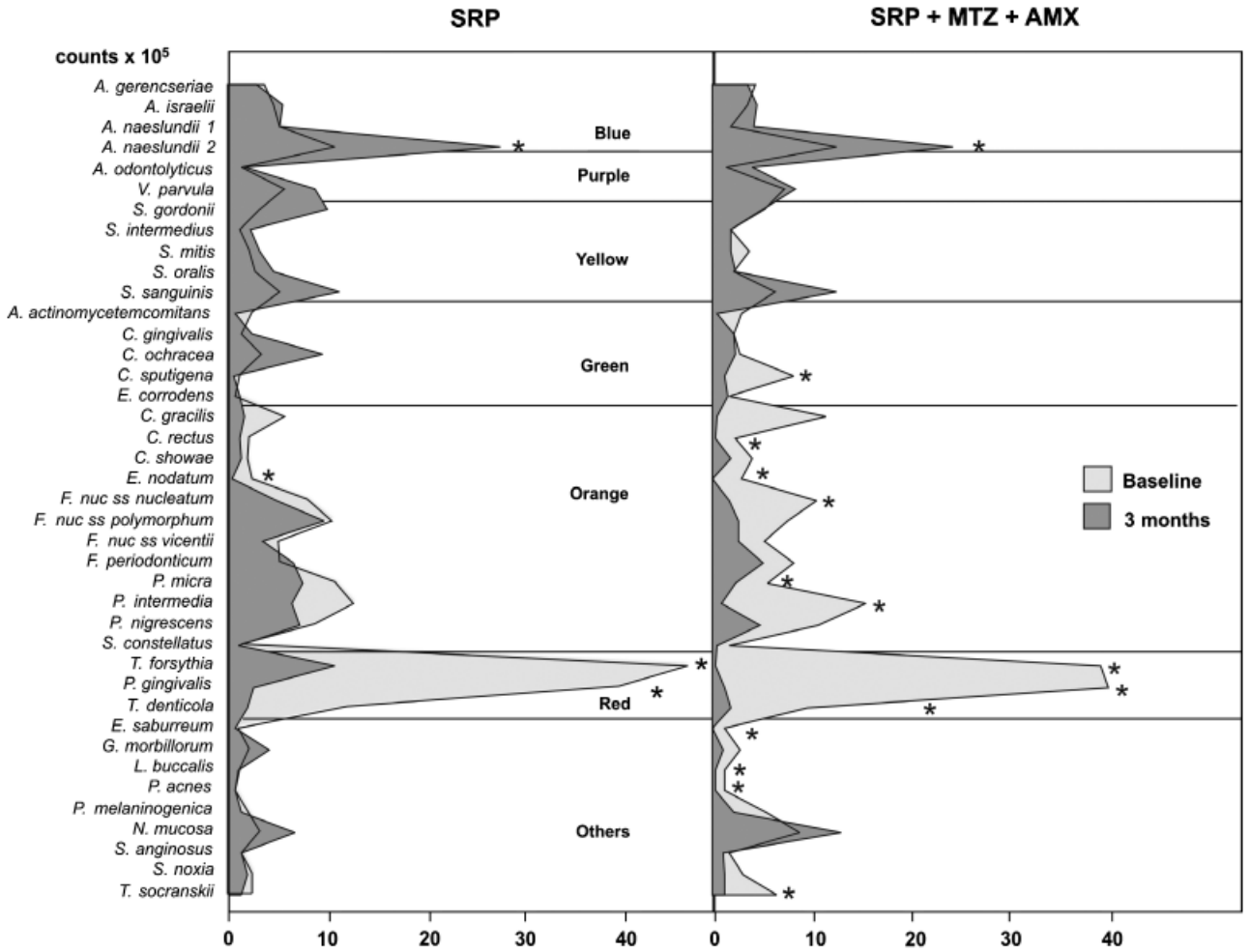


*Fig. 2.* Mean counts ($\times 10^5$) of the 40 test species at baseline and 3 months post-SRP in the two treatment groups. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The counts of individual species were averaged within a subject and then across subjects in each treatment group at each time point. The significance of differences between baseline and 3 months post-SRP was assessed using the Wilcoxon test ($^*p<0.05$), and adjusted for 40 comparisons (Socransky et al. 1991). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

#### Clinical findings

The demographic characteristics and the full-mouth mean values of periodontal clinical parameters at baseline and at 3 months after treatments are presented in Table 3. No statistically significant differences were observed between groups for any parameter at baseline ($p>0.05$). All therapies led to a statistically significant decrease in the mean PD, CAL and in the percentage of sites with visible plaque, gingival bleeding, BOP and suppuration. At 3 months post-SRP, the full-mouth mean PD was significantly lower in the Test group in comparison with the Control group (Table 3) ($p<0.05$). The mean changes in PD and CAL between baseline and 3 months post-therapy are presented in Fig. 6. These two parameters were more strikingly reduced by the antibiotic therapy ($p<0.05$; Fig. 6).

In order to better understand the effect of the therapies and to allow more comprehensive comparisons between the groups, the sites were subset into baseline PD categories of shallow ($\leqslant 3$ mm), intermediate (4–6 mm) and deep ($\geqslant 7$ mm), and the statistical analysis was repeated (Fig. 7). The combination of MTZ and AMX was more effective than SRP alone in improving PD and CAL in all the categories of PD. All these differences were statistically significant, except for CAL in initially shallow sites.

Figure 8 presents changes in the mean full-mouth CAL for individual subjects from all treatment groups at 3 months post-SRP. The median of CAL change in the 30 subjects of the study was 0.96 mm. The number of subjects showing CAL gain within or above this value (0.96–2.12) was 12 and 3 in the Test and Control groups, respectively. Conversely, the number of subjects presenting CAL change below 0.96 (0.92 to $-0.04$) was 3 and 12 for the Test and Control groups, respectively.

Table 4 presents the mean percentage of sites with PD $<5$ or $\geqslant 5$ mm at baseline and at 3 months post-treatments. The two groups were homogeneous for these PD categories at baseline ($p>0.05$). The percentage of sites with PD $<5$ mm increased significantly, and those with PD $\geqslant 5$ mm significantly decreased in both treatment groups over the course of the study. However, at 3 months post-treatment, the Test group showed significantly fewer sites with PD $\geqslant 5$ mm and more sites with

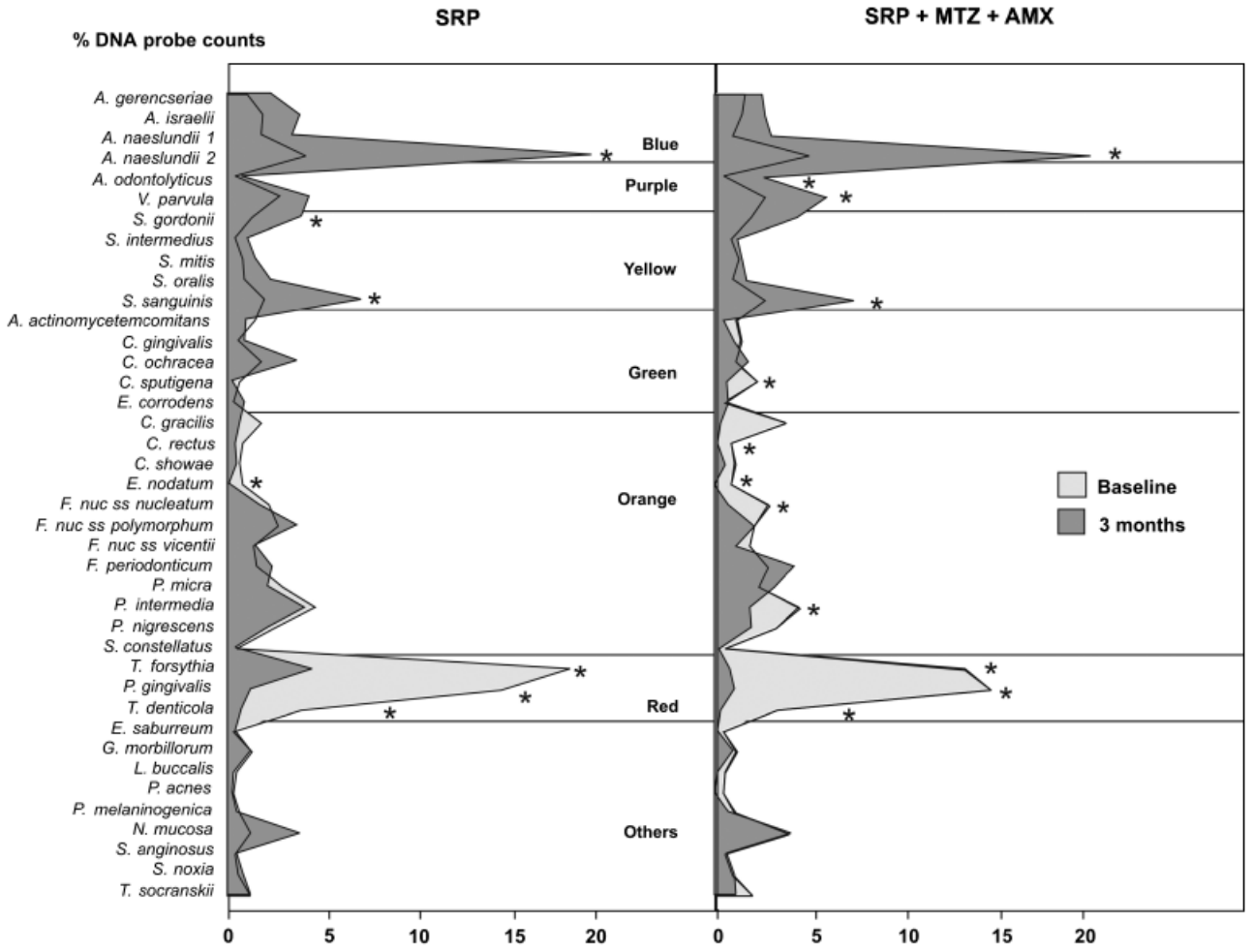


*Fig. 3*. Mean percentage of DNA probe counts of the 40 test species at baseline and 3 months post-therapy in the two treatment groups. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The proportion of each species in the total DNA probe count was determined at each site, and then averaged within and across subjects in each treatment group at each time point. Significance testing is as described in Fig. 2. SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

PD < 5 mm than the Control group. The changes observed in the mean percentage of sites with PD < 5 mm or ⩾ 5 mm between baseline and 3 months were more striking in the Test group in comparison with the Control group.

## Discussion

Previous studies have reported additional clinical benefits when systemic antibiotics, in particular the combination of MTZ and AMX, are implemented as part of the periodontal treatment of subjects with GAgP (Guerrero et al. 2005, Xajigeorgiou et al. 2006, Kaner et al. 2007, Machtei & Younis 2008). Nonetheless, the effect of this therapeutic protocol on the microbial profile has not yet been established. Therefore, to our knowledge, the present study is the first RCT that has systematically evaluated changes occurring in the subgingival microbiota with the use of MTZ and AMX in association with SRP for the treatment of non-smoker subjects with GAgP.

The Test group presented the most favourable changes in the subgingival microbial profile after treatment. The levels and proportions of the red complex pathogens (*T. forsythia*, *P. gingivalis* and *T. denticola*) were more strikingly reduced when the antibiotics were used. At 3 months post-SRP, the proportion of this complex was significantly reduced in the Test group (2.8%) in comparison with the Control group (8.1%) (Fig. 4). In addition, this therapy led to a significant reduction in the proportions of the putative pathogens from the orange complex, which was not observed in subjects who received only SRP (Fig. 4). The only species from this complex affected by SRP alone was *E. nodatum* (Figs 2 and 3). These results are in agreement with the data reported by Xajigeorgiou et al. (2006), who also observed a significant reduction in the levels of the three species from the red complex 6 months after the administration of MTZ and AMX.

*A. actinomycetemcomitans* is an important periodontal pathogen implicated in the aetiology of AgP (Slots 1976, Yang et al. 2004, Fine et al. 2007, Haubek et al. 2008, Faveri et al. 2009). There is a general agreement that SRP alone cannot eliminate or strikingly suppress the levels of this pathogen in subjects with periodontal disease (Renvert et al. 1990, Takamatsu et al. 1999, Mombelli et al. 2000). Winkel et al. (1998) reported the beneficial effects of the combination of MTZ and AMX in the treatment of subjects colonized by *A. actinomycetemcomitans* and Pavicić

et al. (1992, 1994) suggested a possible synergistic effect of this combination of drugs in inhibiting this periodontal pathogen. Therefore, the combination of AMX and MTZ was originally proposed for the elimination or the suppression *A. actinomycetemcomitans* (van Winkelhoff et al. 1989). Overall, in the present study, the two therapies did not significantly change the mean levels and proportion of *A. actinomycetemcomitans* when samples from all PD categories were analysed (Figs 2 and 3). However, when the samples were divided according to the initial PD categories, the Test group presented a significant reduction in the levels of *A. actinomycetemcomitans* in initially deep sites (⩾7 mm) compared with the Control group. In addition, there was a significantly lower mean percentage and number of subjects colonized by *A. actinomycetemcomitans* in the Test group in comparison with the Control group ($p<0.05$) at 3 months post-therapy (Tables 1 and 2). These results are in agreement with previous studies showing that the success of periodontal therapy in subjects with AgP was associated with the reduction of this bacterial species (Haffajee et al. 1984, Mandell et al. 1986; Xajigeorgiou et al. 2006). Nevertheless, it is important to note that only four subjects in the test group were negative for *A. actinomycetemcomitans* after therapy. These results differ from previous studies that showed complete suppression of this pathogen in all subjects treated with SRP plus MTZ and AMX (van Winkelhoff et al. 1989, Berglundh et al. 1998, Winkel et al. 2001). However, in agreement with our data, Xajigeorgiou et al. (2006) did not observe complete elimination of *A. actinomycemtecomitans* in 10 deep periodontal sites of GAgP subjects treated with this combination of drugs. The authors treated 10 subjects with GAgP with AMX plus MTZ and only one subject tested negative for *A. actinomycemtecomitans* at approximately 5 months after the antibiotic treatment. Likewise, Cortelli et al. (2009) chose 50 subjects colonized by *A. actinomycemtecomitans* and treated them with SRP plus MTZ, AMX and periodontal surgery. The authors reported that only 18 subjects tested negative for *A. actinomycemtecomitans* 12 months post-therapy. Some hypothesis could be raised to explain these conflicting results regarding the elimination of *A. actinomycetemcomitans*. The first possibility to be considered would be differences in the microbiological test used by different authors, such as bacterial culture (van Winkelhoff et al. 1989, Berglundh et al. 1998, Winkel et al. 2001), polymerase chain reaction (PCR) (Cortelli et al. 2009) and checkerboard DNA–DNA hybridization (Xajigeorgiou et al. 2006) and the present study). However, it is important to highlight that these three diagnostic tests have excellent (PCR) to very good (Bacterial culture and checkerboard DNA–DNA hybridization) sensitivity, and therefore they would all be effective in detecting *A. actinomycetemcomitans*. It should also be considered that variations in the composition and in

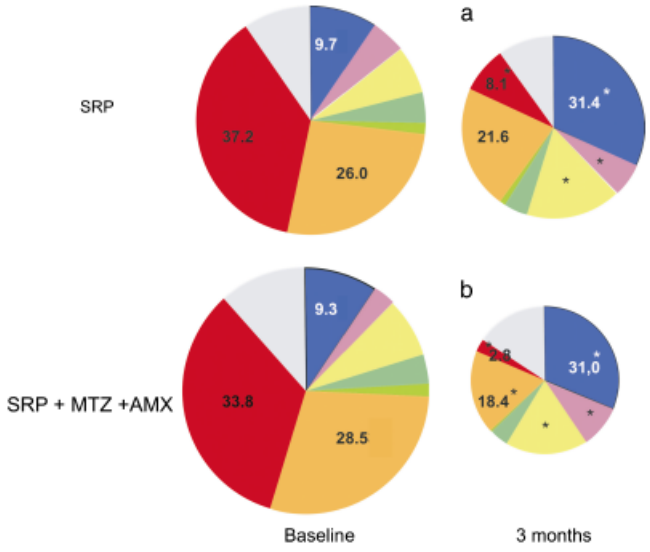


*Fig. 4*. Pie charts of the mean proportion of each microbial complex at baseline and 3 months post-SRP in the two treatment groups. The colours represent different microbial complexes (Socransky et al. 1998). The areas of the pies were adjusted to reflect the mean total counts at each time point. The significance of differences between baseline and 3 months was assessed using the Wilcoxon test ($^*p<0.05$). The significance of differences between treatment groups at baseline and 3 months post-therapy was assessed using the Mann–Whitney *U*-test ($p<0.05$; different letters indicate statistically significant differences). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

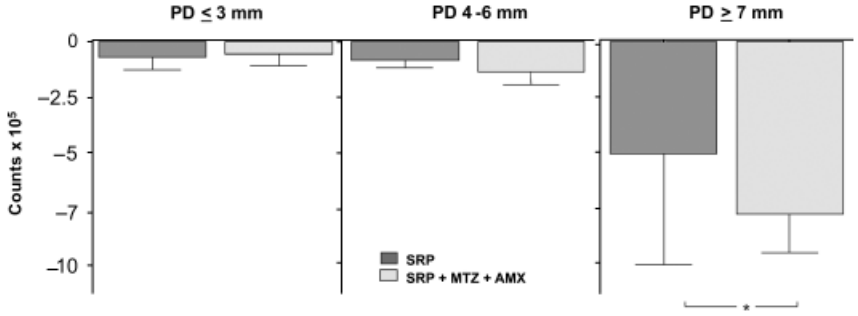


*Fig. 5*. Bar charts of the mean changes in mean levels ($\times 10^5$) of *Aggregatibacter actinomycetemcomitans* at sites with initial probing depths ⩽3, 4–6 and ⩾7 mm between baseline and 3 months post-SRP in the two treatment groups. The whiskers represent the SD. The significance of difference between the two treatment groups was assessed using the Mann–Whitney *U*-test ($^*p<0.05$). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

the complexity of the subgingival microbiota (Haffajee et al. 2004, 2005) as well as in the antibiotic susceptibility of periodontal pathogens (van Winkelhoff et al. 2005) in distinct geographic locations may also contribute to differences in the effectiveness of systemic antibiotics.

The proportions of the host-compatible species were also affected by treatments. Both groups showed a significant increase in the proportions of the purple, yellow and especially in the *Actinomyces* species (blue complex) at 3 months after SRP. It has been suggested previously that in addition to the reduction in pathogens, an increase in beneficial species is necessary to achieve a successful treatment outcome (Teles et al. 2006). This may be an even more important requirement when dealing with AgP. In a recent study conducted by our group (Faveri et al. 2009), the subgingival microbial composition of subjects with localized AgP and GAgP was evaluated and compared with that of periodontally healthy individuals or those with chronic periodontitis. Subjects with AgP presented reduced proportions of the host-compatible *Actinomyces* species compared with those who had chronic periodontitis. The low proportions of this microbial group (around 9%) were also confirmed in the present investigation. Therefore, increased proportions of this microbial group may be necessary for a successful therapeutic outcome. It should be noted that the increase in the proportions of the *Actinomyces* species observed in both treatment groups in the present investigation was more striking than that observed in other studies that evaluated the effect of these therapies in chronic periodontitis subjects (Carvalho et al. 2005, Matarazzo et al. 2008). This effect may be attributed to the CHX rinsing that was used by all subjects in the present study. Feres et al. (2009) have shown that the use of this antiseptic during the SRP and the healing phase may cause a drastic increase in the proportions of *Actinomyces* species at 3 months post-therapy.

Both treatments used in the present study improved the majority of the clinical parameters evaluated. However, in agreement with the microbiological findings, the combination of MTZ and AMX with SRP yielded significant clinical benefits over SRP alone. Overall, benefits of the combined treatments were observed in the full-mouth data analyses (Table 3 and Fig. 6) and in all initial PD categories, especially in the intermediate (4–6 mm) and deep ($\geqslant$7 mm) sites (Fig. 7). In addition, the individual changes in mean CAL after treatments (Fig. 8) re-inforce the benefits of the test treatment also at a subject level. Another important observation of the present study was the lower percentage of sites with PD$\geqslant$5 mm detected after treatment in the Test group, in comparison with the Control group (Table 1). Renvert & Persson (2002) reported that the presence of deep residual pockets after treatment was associated with further disease progression. In addition, Matuliene et al. (2008) stated that more than eight sites with PD$\geqslant$5 mm represent a risk factor for additional attachment or tooth loss with an odds ratio of 5.8. Therefore, residual sites with PD$\geqslant$5 mm could be used as a measure of ''need for further treat-

*Table 1*. Mean percentage of sites colonized by *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola* at baseline and 3 months post-SRP in the two treatment groups

| Variable | Time point | Treatment groups | |
|---|---|---|---|
| | | SRP ($n = 15$) | SRP+MTZ+AMX ($n = 15$) |
| *A. actinomycetemcomitans* | Baseline | 62.6 ± 28.0[a] | 61.4 ± 31.0[a] |
| | 3 months* | 35.6 ± 27.6[a] | 14.0 ± 14.8[b] |
| *T. forsythia* | Baseline | 81.6 ± 28.0[a] | 77.7 ± 29.8[a] |
| | 3 months* | 40.4 ± 33.3[b] | 5.9 ± 10.1[b] |
| *P. gingivalis* | Baseline | 84.0 ± 17.8[a] | 81.3 ± 25.3[a] |
| | 3 months* | 58.6 ± 27.7[b] | 35.5 ± 30.8[b] |
| *T. denticola* | Baseline | 75.3 ± 27.6[a] | 69.5 ± 24.8[a] |
| | 3 months* | 38.0 ± 33.9[b] | 13.2 ± 13.4[b] |

The significance of differences between time points was assessed using the Wilcoxon test (different small letters indicate $p<0.05$). Significance of difference between groups was assessed using the Mann–Whitney *U*-test ($^{*}p<0.05$).

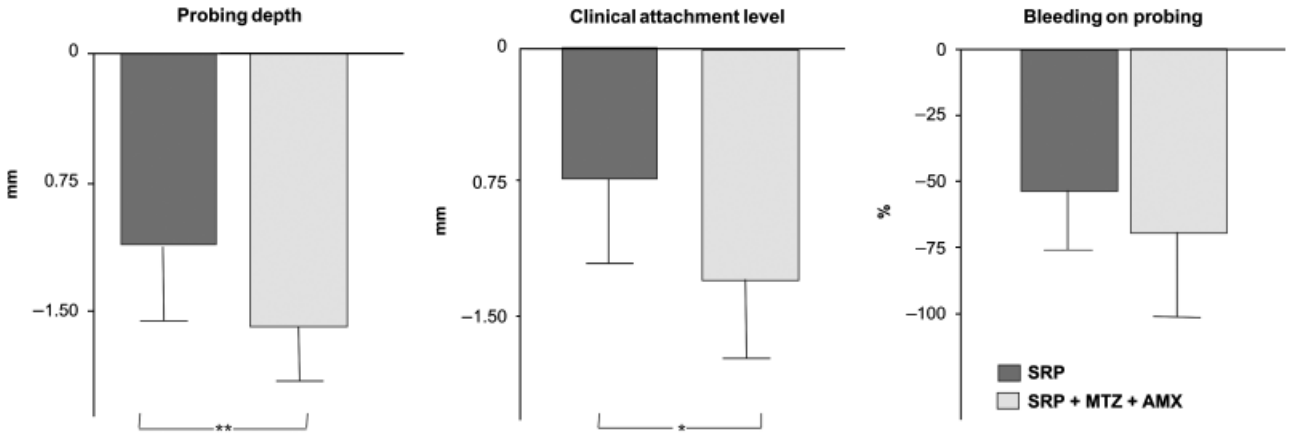


*Fig. 6*. Bar charts of the mean changes (± SD) in full-mouth probing depth, clinical attachment level and percentage of sites with bleeding on probing between baseline and 3 months post-SRP in the two treatment groups. The whiskers represent the SD. The significance of difference between the two treatment groups for each clinical parameter was assessed using the Mann–Whitney *U*-test ($^{*}p<0.05$; $^{**}p<0.01$). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

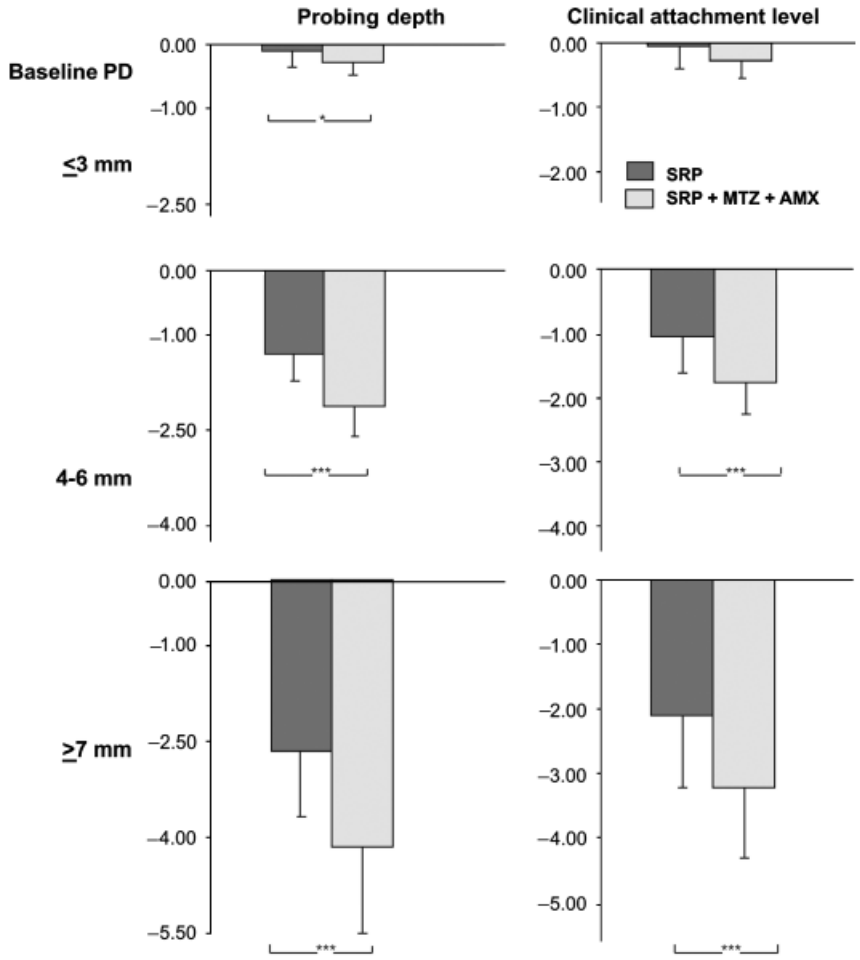


*Fig. 7.* Bar charts of the mean changes (± SD) in probing depth and clinical attachment level at sites with initial probing depths ⩽3, 4–6 and ⩾7 mm between baseline and 3 months post-SRP in the two treatment groups. The whiskers represent the SD. The significance of difference between the two treatment groups for each clinical parameter was assessed using the Mann–Whitney *U*-test ($^{*}p<0.05$; $^{***}p<0.001$). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

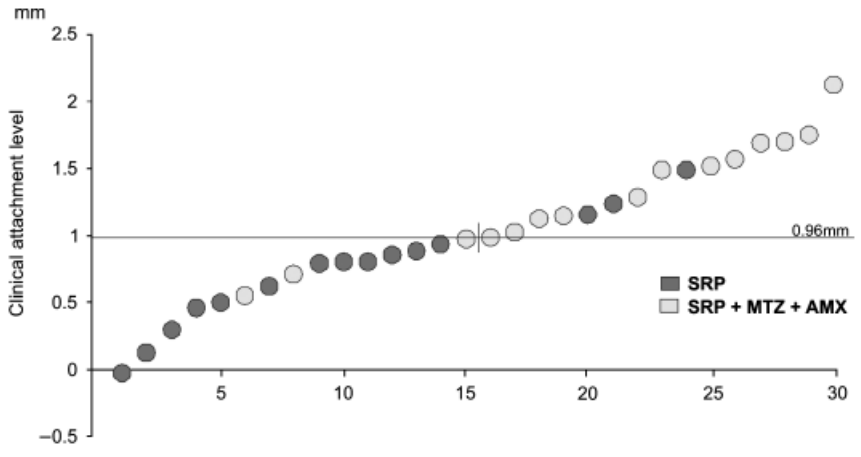


*Fig. 8.* Plots of the mean changes in individual full-mouth mean clinical attachment level between baseline and 3 months post-SRP of subjects in the two treatment groups. The circles represent the mean value of each subject. The dashed line represents the median of change of this clinical parameter in all 30 subjects. Positive values represent a gain in clinical attachment level (CAL), while negative values represent a loss in CAL at 3 months post-SRP. SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

ment''. In the present study, at 3 months post-SRP, the Test group showed a 79% reduction in the number of sites in need of further therapy, while the Control group showed a reduction of 57% (Table 4). Overall, these findings agree with and extend data from previous investigations that also suggested additional clinical benefits when MTZ and AMX are combined with SRP in the treatment of GAgP. (Guerrero et al. 2005, Xajigeorgiou et al. 2006, Kaner et al. 2007, Machtei & Younis 2008).

The clinical efficacy of adjunctive systemic MTZ and AMX compared with SRP alone in GAgP subjects has only been investigated in two RCTs (Guerrero et al. 2005, Xajigeorgiou et al. 2006). In these two reports, at 6 months post-SRP, the combination of SRP plus AMX and MTZ resulted in a mean full-mouth reduction of 1.2 and

*Table 2*. Frequency of subjects colonized by *Aggregatibacter actinomycetemcomitans* at baseline and 3 months post-SRP in the two treatment groups

| Number of sites colonized | Number of subjects colonized by *A. actinomycetemcomitans* | | | |
|---|---|---|---|---|
| | control | test | control | test |
| | baseline | baseline | 3 months | 3 months |
| 0 | 0 | 0 | 2 | 4 |
| 1 | 1 | 1 | 2 | 8 |
| 2–4 | 3 | 5 | 7 | 3 |
| ⩾5 | 11 | 9 | 4 | 0 |

Control, SRP; Test, SRP+MTZ+AMX.

*Table 3*. Demographic characteristics and mean (± SD) full-mouth clinical parameters at baseline and 3 months post-therapy in the two treatment groups

| Variable | Time point | Treatment groups | |
|---|---|---|---|
| | | SRP ($n = 15$) | SRP+MTZ+AMX ($n = 15$) |
| Gender (male/female) | Baseline | 4/11 | 6/9 |
| Age (years) | Baseline | 27.6 ± 3.5 | 26.8 ± 3.9 |
| % sites with | | | |
| PD⩽3 mm | Baseline | 47.8 ± 17.3 | 51.7 ± 11.7 |
| PD 4–6 mm | Baseline | 37.5 ± 16.8 | 31.9 ± 10.9 |
| PD⩾7 mm | Baseline | 14.7 ± 8.3 | 16.4 ± 12.3 |
| PD (mm) | Baseline | 4.1 ± 0.6[a] | 4.3 ± 0.7[a] |
| | 3 months* | 3.2 ± 0.6[b] | 2.7 ± 0.5[b] |
| CAL (mm) | Baseline | 4.2 ± 0.5[a] | 4.5 ± 0.8[a] |
| | 3 months | 3.5 ± 0.5[b] | 3.2 ± 0.5[b] |
| % of sites with | | | |
| Plaque accumulation | Baseline | 62.7 ± 22.4[a] | 61.3 ± 19.8[a] |
| | 3 months | 34.8 ± 16.5[b] | 34.3 ± 15.0[b] |
| Gingival bleeding | Baseline | 23.7 ± 20.4[a] | 37.3 ± 27.2[a] |
| | 3 months | 5.0 ± 6.5[b] | 3.8 ± 0.4[b] |
| Bleeding on probing | Baseline | 63.8 ± 21.3[a] | 77.7 ± 19.7[a] |
| | 3 months | 12.5 ± 11.7[b] | 12.2 ± 13.0[b] |
| Suppuration | Baseline | 3.8 ± 9.3[a] | 1.8 ± 3.8[a] |
| | 3 months | 0.2 ± 0.1[b] | 0 ± 0[b] |

The significance of differences between time points was assessed using the Wilcoxon test (different small letters indicate $p < 0.05$). Significance of the difference between groups was assessed using the Mann–Whitney *U*-test (*$p < 0.05$). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin; PD, probing depth; CAL, clinical attachment level.

*Table 4*. Mean percentage and number of sites with PD<5 mm or ⩾5 mm at baseline and at 3 months post-therapy

| % Sites with (number of sites) | Time point | Treatment groups | |
|---|---|---|---|
| | | SRP | SRP+MTZ+AMX |
| PD<5 mm | Baseline | 69.1 ± 11.2 [a] | 62.3 ± 12.4 [a] |
| | | (95.3 ± 15.8) | (89.7 ± 17.2) |
| | 3 months* | 86.8 ± 9.3 [b] | 92.1 ± 8.5[b] |
| | | (119.8 ± 12.8) | (132.6 ± 12.1) |
| PD⩾5 mm | Baseline | 30.9 ± 11.1[a] | 37.7 ± 12.5 [a] |
| | | (42.7 ± 15.4) | (54.3 ± 17.3) |
| | 3 months* | 13.2 ± 9.4[b] | 7.9 ± 8.5[b] |
| | | (18.2 ± 15.4) | (11.4 ± 12.2) |

The significance of differences between time points was assessed using the Wilcoxon test (different small letters indicate $p < 0.05$). Significance of the difference between groups was assessed using the Mann–Whitney *U*-test (*$p < 0.05$). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin; PD, probing depth.

1.5 mm in PD, and a mean CAL ''gain'' of 0.8 and 0.9 mm, respectively. In the present study, the Test group showed a reduction of 1.6 mm in PD and a CAL gain of 1.3 mm. In addition, in initially deep sites (PD⩾7 mm), the mean CAL gain at 3 months was 2.0 and 3.2 mm for the Control and Test groups, respectively. Beneficial results were also observed in the initially intermediate and shallow sites. Overall, these results are somewhat better than those reported by Guerrero et al. (2005) and Xajigeorgiou et al. (2006), even for the subjects in the Control group. One possible explanation for these differences is the fact that the studies differ to some extent regarding the treatment protocols used. It should be highlighted that in the present study, both groups rinsed with 0.12% CHX for 2 months. Indeed, previous studies demonstrated the clinical and microbiological benefits of the optimal supragingival plaque control using CHX rinses during and after SRP in subjects with chronic periodontitis (Faveri et al. 2006, Feres et al. 2009).

In addition to the beneficial clinical and microbiological results observed in the present study, it was also interesting to observe that only two subjects (6.6%, one from the Control and one from the Test group) presented adverse events. This result is in accordance with a previous study from our group that used the same antibiotic protocol in the treatment of smokers with chronic periodontitis (Matarazzo et al. 2008).

One limitation of the current study is the short-term evaluation period. Indeed, longitudinal monitoring of these subjects will be important in order to determine whether this combination of therapies would produce sustained beneficial changes in the subgingival microbial profile and periodontal clinical parameters over time. Nevertheless, it has been suggested that the short-term changes in the microbial profile may determine long-term periodontal clinical stability (Winkel et al. 2001, Haffajee 2003, Matarazzo et al. 2008). Because the effects of this antibiotic protocol on the subgingival microbial profile are not yet known, the 3-month data presented in this manuscript provide important information for the periodontal literature.

In conclusion, the use of MTZ and AMX in combination with SRP yields short-term additional clinical and microbiological advantages in the treatment of GAgP.

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**Clinical Relevance**

*Scientific rationale for the study:* It is generally accepted that the association of MTZ plus AMX with SRP benefits the treatment of GAgP subjects. However, only a few controlled clinical trials to date have addressed this topic, and the microbial effects of this treatment have not yet been investigated.

*Principal findings:* Subjects receiving systemic antibiotics showed additional beneficial changes in the composition of the subgingival microbiota and in the clinical parameters in comparison with those receiving SRP only.

*Practical implications:* The more striking effects of MTZ plus AMX in the subgingival microbial profile leads the short-term clinical advantages in the treatment of GAgP.